

Full Length Research Paper

Hypoglycaemic and hypolipidemic effects of pumpkin (*Cucurbita pepo* L.) on alloxan-induced diabetic rats

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This study was aimed to evaluate the hypoglycaemic and hypolipidemic effects of different doses of pumpkin (*Cucurbita pepo* L.) powder in male diabetic rats. A total of 35 rats were randomized into 5 groups of 7 each as follows: Group 1: Normal control; Group 2: Diabetic control; Group 3: Diabetics administered with low doses of pumpkin powder (1 g/kg); Group 4: Diabetics administered with high doses of pumpkin powder (2 g/kg), and Group 5: Diabetics administered with glibenclamide (0.6 mg/kg), as positive control. The rats were made diabetic by alloxan (120 mg/kg body weight (BW)) injection and were treated for 4 weeks on a daily basis. Blood samples were collected following the experiment. Pancreatic specimens were also collected for histological analysis. Glucose, cholesterol, triglycerides, low density lipoprotein (LDL) and C-reactive protein (CRP) were significantly increased, while insulin was decreased in diabetic rats as compared to the normal control group ($p < 0.05$). Low dose pumpkin significantly decreased glucose, triglycerides, LDL and CRP as compared to diabetic group and high dose pumpkin decreased cholesterol ($p < 0.05$). Histological analysis also revealed a significant increase in the diameter and number of langerhans islets in treated group with pumpkin, which was consistent with the latter findings. Therefore, pumpkin might be beneficial in diabetic patients.

Key words: *Cucurbita pepo*, diabetes, pumpkin, alloxan monohydrate, rat, hypoglycaemic, hypoglycaemic.

INTRODUCTION

Diabetes mellitus is a chronic disorder of carbohydrate, lipid and protein metabolism manifested by elevated blood glucose level. This disease is caused by a defect in cellular uptake of glucose due to either reduced insulin secretion or cellular resistance to insulin (DeFronzo, 1997; Hughs et al., 1984). Clinically, diabetes is an important risk factor for a range of diseases including nephropathy, retinopathy and neuropathy, and it is increasing in prevalence according to some estimates (Tripathi and Srivastava, 2006). In addition, lipid

disorders and lipid per-oxidation together with diabetes play a crucial role in the development of cardiovascular disease (David et al., 2005; Roland et al., 2004). Although, insulin and hypoglycaemic drugs constitute the main treatment in diabetes, the use of nutritional methods and medicinal plants are increasing in some countries (Grover et al., 2002; Khan and Anderson, 2003).

Pumpkins (genus; *Cucurbita*) belong to the family of Cucurbitaceae. They are classified as *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita mixta* according to the texture and shape of their stems. This family contains chemicals, including tetra cyclic triterpens, saponins, proteins, fibers, polysaccharides and minerals (iron, zinc, manganese, copper, etc)

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(Bombardelli and Morazzoni, 1997; Lazos, 1986). Pectin, a major component of plant cell walls, it is a water-soluble fiber found abundantly in pumpkin plants (Fissore et al., 2009). The seeds of this plant, which have been implicated in providing many health benefits, are rich natural source of fatty acids (including linoleic acid, oleic acid, palmitic acid and stearic acid), phenolic compounds (Applequist et al., 2006; Xanthopoulou et al., 2009) and also, antioxidant vitamins, such as carotenoids and tocopherol (Stevenson et al., 2007). So far, several pharmacological properties have been reported for different species of pumpkin including anti-oxidant, lipid-lowering, hepatoprotective (Makni et al., 2008), anti-carcinogenic (Hong, 2005), anti-microbial (Park et al., 2010) and anti-diabetic properties (Caili et al., 2006; Xia and Wang, 2006). Therefore, the aim of this study was to investigate the effects of various doses of *C. pepo* on elevated blood glucose and lipid levels and the histological changes of pancreatic tissue in type 1 diabetes in male rats.

MATERIALS AND METHODS

Preparation and standardization of pumpkin powder

The fruit pumpkin (*C. pepo* L.) was cultivated in a farm in Isfahan in April 2008. The genus and species was confirmed by technicians of the Botany Department of Isfahan University and a voucher specimen (No.1400) was kept in the herbarium of the Faculty of Pharmacy, Isfahan University of Medical Sciences. The fruits were cut and dried in well-ventilated stores under standard conditions away from sunlight, moisture and microbial contamination. The plants were then powdered using electric grinders.

The pumpkin has been analyzed for the presence of phytochemicals (Mukesh et al., 2010). However, because the proportion of each phytochemical in plants of same species vary in different regions, we standardized the pumpkin by measuring the amounts of its phenolic and polysaccharide compounds as well as antioxidant activity.

Measurement of total phenolic compounds

The amount of total phenolic compounds in pumpkin was determined colorimetrically using the Folin-Ciocalteu reagent, by Francis (1982) method. In brief, 5 ml of pumpkin extract or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M). The mixtures were allowed to stand for 15 min, and the total phenols were determined by colorimetry at 765 nm. A standard curve was prepared using 0, 50, 100, 150, 200 and 250 mg/L solutions of gallic acid in methanol:water (50:50, v/v). Total phenol values were expressed in terms of gallic acid equivalent (mg/g). The experiment was repeated in triplicate.

Measurement of polysaccharides

The Beck and Bibby (1961) anthrone technique was employed for polysaccharide determination with some modifications. Briefly, 4 or 5 ml of the diluted samples of pumpkin extract was added to 0.5 ml of the 2% anthrone-ethyl acetate reagent in suitable test tubes. 5 ml of 96.7% sulfuric acid were then carefully layered into each tube.

The contents of the tube were then thoroughly mixed by more rapid swirling to redissolve the anthrone in the sulfuric acid and it was left in the boiling water for a total of 10 min. They were then transferred to an ice bath for 5 min. The samples were transferred to optical tubes and read in a colorimeter using a 630 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) filter. Glucose with dilutions of 0 to 100 μl was used to prepare standard curve. Each sample was read three times, the 100 per cent transmittance being checked and adjusted if necessary with the distilled water-reagent-sulfuric acid blank between each reading. The means of the three readings were used to derive optical density values, which were substituted in the regression equation to give the carbohydrate concentrations. The mean of the three aliquots was taken as the final carbohydrate value.

Evaluation of antioxidant activity

Antioxidant activity of the extract samples was determined using the ferric thiocyanate method (Masude et al., 1992). In this method, 500 μg of each sample was dissolved in ethanol and was added to a reaction mixture containing 2.88 ml of 2.5% linoleic acid and 9 ml of 40 mM phosphate buffer in a vial. The vials were incubated at 40°C for 96 h. During incubation (every 12 h), 0.1 ml of each vial was diluted with 9.7 ml of 75% ethanol, 0.1 ml of ammonium thiocyanate and 0.1 ml of FeCl_2 . The absorbance of samples was measured at 500 nm, and the percentage inhibition (the capacity to inhibit the peroxide formation in linoleic acid) was determined using the following equation:

$$\text{Percentage of inhibition} = (1 - [\text{absorbance of sample} / \text{absorbance of control}]) \times 100$$

A high inhibition percentage indicates a high antioxidant activity. Ethanol within the sample and without reagents was used as the negative control.

Laboratory animals

In the present experiment, white male Wistar rats (*Ratus norvegicus* allivias) in the range of 180 to 220 g body weight were provided by Ahvaz Medical University. The animals were kept under standard conditions in Esfahan University's animal house and had free access to water and food. In order for the animals to adapt to the new environment all experiments were carried out 2 weeks after their accommodation. All of the animal experimental procedures validated by the local ethical committee of the Shahrekord University of Medical Sciences (No: 1389/209).

Induction of diabetes

Type 1 diabetes was induced using intraperitoneal injection of alloxan monohydrate (120 mg/kg) (Sigma, Germany) which was dissolved in normal saline immediately before usage (Venkatesh et al., 2010; Madhavan et al., 2008). Diabetes was verified in the animal by measuring fasting blood glucose 3 days following alloxan injection (Ragavan and Krishnakumari, 2006; Rajagopal and Sasikala, 2008). Rats with glucose levels over 130 mg/dl were considered diabetic (Sharma et al., 2010; Quanhong et al., 2005).

Categorization

A total of 35 rats were randomized into 5 groups of 7 each: group 1: normal control; Group 2: Diabetic control; Group 3: Diabetic rats treated with pumpkin powder (1 g/kg) by gavage; Group 4: Diabetic

rats treated with pumpkin powder (2 g/kg) by gavage, group 5: diabetic rats treated with reference drug, glibenclamide (0.6 mg/kg/day) for 4 weeks, as positive control.

Blood sampling and biochemical tests

Following the 4-week period, blood samples were collected and the levels of glucose, insulin, plasma lipid and lipoprotein and CRP were measured. The animals were deprived of food for 16 h before blood sampling (Ragavan and Krishnakumari, 2006; Asgary et al., 2008). Blood samples were kept at room temperature for 40 min and then subjected to centrifugation at 3000 rpm for 15 min and was utilized for biochemical studies (Quanhong et al., 2005).

The concentration of cholesterol, triglyceride, low density lipoprotein (LDL), high density lipoprotein (HDL) and C-reactive protein (CRP) was measured using commercially available kit obtained from Pars Azmoon (Iran) which utilized the colorimetric method (Rifai et al., 1999; Thompson et al., 1992). Plasma glucose levels were estimated by an enzymatic glucose oxidase-peroxidase (GOD-POD) method using commercially available kit obtained from BioSystems (Iran) (Trinder, 1969). Plasma insulin was determined using Monobind kit and Enzyme Linked Immunosorbant Assay (ELISA) method (Turkington et al., 1982).

Histological examinations

At the end of the experiment the rats were euthanized and the pancreas was removed. The specimens were washed with normal saline and were fixed in 10% formalin. The tissues were then dehydrated and cut into 5 micron sections and were stained with hematoxylin and eosin. Prepared slides were examined for mean diameter and number of Langerhans islets under the light microscope (Asgary et al., 2008).

Statistical analysis of data

Analysis of data was performed using the SPSS statistical software, version 11, with one way ANOVA. The differences among means were tested for significance using least significant difference (LSD) post hoc test. The criterion for statistical significance was $p < 0.05$. Data are presented as means with \pm SE.

RESULTS

Polysaccharides, phenolic compounds and antioxidant activity

The amount of polysaccharides in dried pumpkin was estimated to be 43.65 mg/g equivalent to glucose and the amount of phenolic compounds was 12 mg/g equivalent to gallic acid. The antioxidant activity (the percentage inhibition or the capacity to inhibit the peroxide formation in linoleic acid) of pumpkin was estimated to be 14%.

Comparison of groups with respect to glucose and insulin levels

Serum glucose levels were found to be significantly higher in diabetic control group as compared to normal

control group. Similar to glibenclamide, consumption of 1 and 2 g/kg pumpkin powder significantly reduced glucose levels in diabetic rats as compared to diabetic control group ($p < 0.05$) (Table 1). Insulin levels were shown to be significantly lower in diabetic control group as compared to normal control group. Pumpkin powder (low and high doses) consumption increased this value, although not significantly, whereas glibenclamide consumption significantly increased insulin levels when compared with diabetic control group ($p < 0.05$) (Table 1).

Comparison of groups with respect to cholesterol and triglyceride levels

It is apparent from the results that cholesterol and triglyceride levels significantly increased in diabetic control group as compared to the normal control group. Administration of high dose pumpkin (2 g/kg) to diabetic rats significantly reduced cholesterol levels as compared to diabetic control group ($p < 0.05$) (Table 1).

Similar to glibenclamide consumption of both high and low dose pumpkin significantly reduced triglyceride levels as compared to diabetic control group ($p < 0.05$) (Table 1).

Comparison of groups with respect to lipoprotein levels

LDL levels were found to be significantly higher in diabetic control group as compared to normal control group.

Consumption of pumpkin powder in diabetic rats lead to a significant decrease in blood LDL. Table 1 illustrates that glibenclamide was not as effective as pumpkin in reducing blood LDL levels and therefore no significant difference was observed between this group and the diabetic control group.

It is evident from the results that reduction of HDL levels in diabetic control group as compared to the normal control group was not significant but administration of glibenclamide to diabetic rats significantly increased this factor as compared to diabetic the control group.

In this regard, there was a significant difference between low and high dose pumpkin, where low-dose pumpkin (1 g/kg) proved to be more effective in increasing this factor in diabetic rats ($p < 0.05$) (Table 1).

Comparison of groups with respect to CRP levels

Results indicate that this factor increases in diabetic control group compared to the other groups and that administration of pumpkin powder (low and high doses) has significantly reduced it to the level seen in normal control group. The group given glibenclamide was not

Table 1. Effect of pumpkin powder on the level of biochemical factors in normal and diabetic rats.

	Normal control	Diabetic control	Pumpkin powder (1 g/kg)	Pumpkin powder (2 g/kg)	Glibenclamide (0.6 mg/kg)
Glucose (mg/dl)	69.71 ± 6.21*	246 ± 30.33 [†]	104.2 ± 10.79*	98.75 ± 25.96*	105.5 ± 8.61*
Insulin (μU/ml)	2.06 ± 0.49*	1.04 ± 0.22 [†]	1.84 ± 0.16	1.55 ± 0.06	2.12 ± 0.49*
Triglycerides (mg/dl)	58.42 ± 9.73*	103.6 ± 22.89 [†]	51.8 ± 4.46*	25.75 ± 6.9*	41.83 ± 8.24*
Total cholesterol (mg/dl)	78.85 ± 4.1*	107.4 ± 5.51 [†]	96.2 ± 8.96	68.5 ± 9.28*	94.83 ± 9.4
LDL (mg/dl)	18.66 ± 1.05*	24.6 ± 1.12 [†]	18 ± 2.46*	16 ± 1.47*	22.66 ± 2.24
HDL (mg/dl)	40.57 ± 1.6	39.8 ± 3.56	49.8 ± 3.33	37.75 ± 4.71 [†]	51.66 ± 4.2* [†]
CRP (mg/dl)	39.71 ± 2.45*	52.4 ± 1.53 [†]	40.8 ± 4.53*	39 ± 1.22*	50.33 ± 1.42 [†]

Values are given as mean ± S.E (n = 7). *Significance difference versus diabetic control (p < 0.05). [†]Significance difference versus normal control (p < 0.05). [‡]Significantly difference versus high dose and low dose of pumpkin powder (p < 0.05).

Table 2. Effect of pumpkin powder on size and number of Langerhans islets in normal and diabetic rats.

	Normal control	Diabetic control	Pumpkin powder (1 g/kg)	Pumpkin powder (2 g/kg)	Glibenclamide (0.6 mg/kg)
Size of islets (micron)	1.3 ± 0.14*	0.5 ± 0.02 [†]	0.87 ± 0.07 [†]	1.06 ± 0.21*	0.91 ± 0.12 [†]
Number of islets	6 ± 0.54*	2.6 ± 0.23 [†]	4.6 ± 0.74*	4 ± 0.4 [†]	4 ± 0.68 [†]

Values are given as mean ± S.E (n=7). *Significance difference versus diabetic control (p < 0.05). [†]Significance difference versus normal control (p < 0.05).

significantly different from diabetic control group in this regard (p < 0.05) (Table 1).

Histological results

Histomorphological analysis shows that the mean diameter and number of the Langerhans islets are different among experimental groups. The mean diameter and number of Langerhans islets (evaluated in 5 micron sections), were significantly reduced in diabetic control group as compared to the normal control group, which was approximately restored to normal when administered with pumpkin powder and glibenclamide. High dose pumpkin (2 g/kg) consumption significantly increased the mean diameter of islets as compared to that of diabetic control group. With respect to the number of islets, significant difference was found between the group fed low dose pumpkin (1 g/kg) and the diabetic control group (Table 2 and Figure 1) (p < 0.05).

DISCUSSION

Results obtained in this study indicated that administration of pumpkin powder for 4 weeks in diabetic rats significantly reduced glucose, cholesterol, triglyceride, LDL and CRP levels as compared to diabetic control group. In addition, this plant was found to increase blood insulin and HDL levels, although this was not significant.

Histological analysis showed significant difference in the mean diameter and the number of Langerhans islets between the diabetic control group and the normal control group. The mean diameter of islets in the diabetic group was significantly decreased as compared to that of the normal control group, which was consistent with other studies (Asgary et al., 2008; Mohajeri et al., 2009). It is evident from the results that the number and mean diameter of pancreatic islets increased in the group fed with pumpkin as compared to the diabetic group which illustrates the effects of pumpkin powder on repair and restoration of pancreatic tissue.

Studies suggest that alloxan selectively destroys pancreatic β cells, therefore making it a suitable drug for induction of experimental diabetes. Its structural resemblance to glucose enables alloxan to enter β cells plasma membrane via glucose transporters (Glut2). Also, alloxan produces re-active oxygen species which is limited to the pancreatic islets (Elsner et al., 2006; Lenzen, 2008). As a result of the higher concentration of plasma, free radicals oxidative stress increases which is of particular importance in the pathogenesis of various diseases, including cardiovascular disease and diabetes (Potapovich and Kostyuk, 2003).

Pumpkin contains various biologically active components, such as polysaccharides, paraaminobenzoic acid, fixed oils, sterols, proteins, peptides, carotenoids, g-aminobutyric acid and vitamins (Murkovic et al., 2002; Matus et al., 1993; Bombardelli and Morazzoni, 1997). The results of this study also showed that pumpkin has

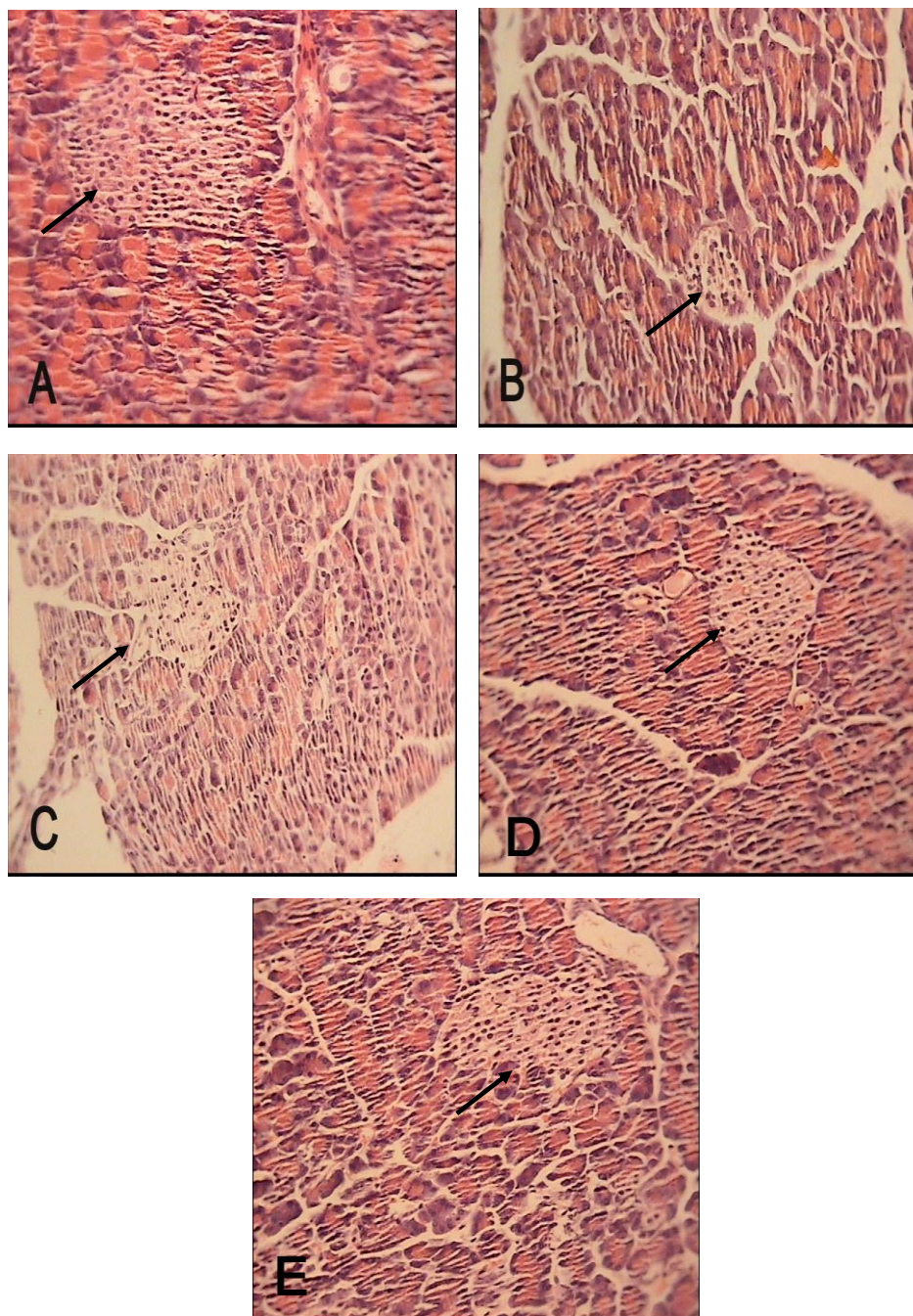


Figure 1. Pancreatic islets in the normal control (A), diabetic control (B), diabetic rats treated with glibenclamide (C), diabetic rats treated with pumpkin powder (1 g/kg) (D) and diabetic rats treated with pumpkin powder (2 g/kg) (E) (40 \times).

that pumpkin has antioxidant activity.

Anti-oxidants are substances that protect cell membranes and other components of an organism against damage caused by oxidants. These compounds function by collecting free radicals, transferring electron to them and ultimately rendering them inactive (Venkat et al., 2006; Vaya and Aviram, 2002). Antioxidant compounds

also increase the number of β pancreatic cells by enhancing the repair and restoration of these cells. Numerous studies have shown that administration of antioxidants to diabetic rats significantly increases the number of β cells (Asgary et al., 2008). Studies have indicated that administration of quercetin to β cells *in vitro*, causes an increase in the number of these cells, which is due to an increase

in DNA replication in pancreatic islet cells (Hii and Howell, 1984). Therefore, the pancreas protective effect of pumpkin and its hypoglycemic properties should be attributable, in part, to antioxidant activity of this fruit. Abdel-Hassan et al. (2000) found that saponin extracted from colocynth (*Citrullus colocynthis*), from the Cucurbitaceae, has high anti-diabetic activities. Flavonoid compounds, including quercetin with antioxidant activity also possess hypoglycaemic effect in diabetic rats (Rauter et al., 2010). Moreover, the presence of pectin itself can serve as a hypoglycaemic agent in pumpkin (Gourgue et al., 1992).

Medicinal plants, such as *Aloe vera* L., *Ocimum sanctum* L. and *Alpinia galanga* (L.), which contain polysaccharides, have been shown to increase the levels of serum insulin, reduce blood glucose level or improve tolerance of glucose (Mukherjee et al., 2006). It has also been found that pumpkin polysaccharides could increase the superoxide dismutase and glutathione peroxidase activity and reduce the malonaldehyde in mice serum which shows an increase in antioxidant capacity (Xu, 2000). The existence of high level of polysaccharides in the examined pumpkin in this study should therefore, be another reason for hypoglycemic activity of pumpkin.

The lipid reducing effects of pumpkin is probably due to its fibres. These substances reduce plasma LDL levels by inhibiting the absorption of bile acids and cholesterol and enhancing the activity of LDL receptors. Furthermore, a fiber-rich diet reduces triglyceride levels by suppressing lipogenesis in the liver (Romero et al., 2002; Lecumberri et al., 2007). The presence of unsaturated fatty acids, such as oleic acid and linoleic acid in pumpkin seed reduce cholesterol levels in rats (Takada et al., 1994). The lipid-reducing properties of this plant are partly attributed to the pectin present in it. Previous data suggest that diets rich in pectin facilitate excretion of bile acids which lead to their synthesis increase from cholesterol in the liver and ultimately reduction of blood cholesterol levels (Fernandez et al., 1990). Pectin enhances the activity of lipoprotein lipase in fat tissue and heart, resulting in higher absorption of triglyceride rich lipoproteins (very low density lipoprotein (VLDL) and chylomicron) in tissues other than liver to promote their breakdown and therefore reducing triglyceride levels (Gomathy et al., 1989). Since cholesterol plays a crucial role in lipoprotein biosynthesis and LDL's contain the highest level of cholesterol, LDL is likely to deplete following a reduction in cholesterol levels. On the other hand, LDL reduction may be due to an increase in LDL catabolism. By regulating LDL receptor gene, flavonoids increase the number of LDL receptors on the surface of liver cells. Following recognition and attachment of LDL apoprotein to LDL receptors, LDL is driven into the hepatocyte and removed from the blood stream (Pal et al., 2003).

With respect to increase in CRP levels in rats our results were consistent with the findings of Goyal et al.

(2008). No reports were found regarding the effects of pumpkin on CPR serum concentration in diabetic rats. It is speculated that the anti-inflammatory effects of this plant is related to its anti-oxidant compounds, such as flavonoids. It is also established that quercetin, from the class of flavonoids, provide protection against free radicals, chelate metal ion transporters and also inhibit oxidases including lipooxygenase (Lean et al., 1999).

By considering all these facts, it can be concluded that pumpkin has potential antidiabetic properties, which may suggest the inclusion of this plant in antidiabetic regimens to treat human diabetes. However, further studies in detail are warranted to explore its active ingredients responsible for the beneficial action and the mechanisms involved. Controlled clinical trials are also strongly needed to confirm the hypoglycemic effects in human subjects. These are our focus for future studies.

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